

## Larval and Early Juvenile Development of the Wavy Turban Snail, *Megastraea undosa* (Wood, 1828) (Gastropoda: Turbinidae)

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**Abstract.** Larval and early juvenile development in *Megastraea undosa* was observed over 70 days under static culture conditions at temperatures ranging from 17°C to 20°C. The larvae were fed *Nannochloropsis oculata* and *Phaeodactylum tricornutum* microalgae. Embryonic development from fertilized egg to competent larval stage lasted 5 days. Teleoconch secretion occurred on day 9. Onset of shell edge crenulations and whorl elevation occurred on days 17 and 30, respectively. Shell pigmentation began on day 45. The growth rate was 14.41  $\mu\text{m}\cdot\text{day}^{-1}$ .

### INTRODUCTION

The use of artificial collectors to estimate invertebrate larvae settlement rates has been regarded as an indicator of recruitment variability at the larval level, particularly in commercial fishery species, such as abalone and lobster (Keesing et al., 1995; Nash et al., 1995; Phillips & Cobb, 2000).

In Baja California, recent experiments have involved artificial settlement collectors to catch abalone larvae (Ponce-Díaz, unpublished data). Samples obtained by this method have revealed many taxa, compelling the authors to expand the scope of their studies on larval recruitment to other gastropods [*Megastraea undosa* (Wood, 1828), *Tegula eiseni* (Jordan, 1936), *T. aureotincta* (Forbes, 1850), and *Megathura crenulata* (Sowerby, 1825)]. These species are co-dominants in the rocky bank habitat where abalone live (Guzmán del Prío et al., 1991).

Attempts to quantify post-larval recruitment of the co-dominant species met with a dearth of literature to assist in their identification at the larval stage. Although *Megastraea* larvae have been used in experiments in some studies (Yool et al., 1986; Carpizo & Rosa-Velez, 1993), there are no descriptions of their larval development. In the case of *Tegula*, Moran (1997) described the development of the species *T. funebris* (Adams, 1855), but no information on this subject was found for *Megathura crenulata*.

Therefore, to understand larval development in these species and acquire reference collections for their identification, the authors performed experimental laboratory culture studies on some of these gastropods. This paper

presents the results of the early development of the wavy turban snail, *Megastraea undosa*, during the first 70 days of development. Some previous studies have referred to this species by the name *Astraea undosa*.

### MATERIALS AND METHODS

#### Collection and Holding of Specimens

In June 2001, 24 adult specimens of *Megastraea undosa* (basal diameter > 90 mm) were collected at Los Morros, Bahía Tortugas, B.C.S. at a depth of 12 m. The specimens were taken to the CIBNOR laboratory near La Paz, B.C.S. in a cooler, maintaining humid conditions by placing the specimens between layers of the macroalgae, *Macrocystis pyrifera*, (L.) C. Agardh at an interior temperature of 10°C.

In the laboratory, the specimens were placed in circular, 1.5 m diameter tanks with a 200 L capacity. Seawater in the tank was kept at 19°C with constant aeration. The specimens were fed rehydrated macroalgae leaves (*Macrocystis pyrifera*), and the water and food were entirely replaced every 2 days.

#### Gonad Conditioning

To attain maximum gonadal development, specimens were fed *ad libitum* and kept at a constant temperature of 19°C, which is the average seawater temperature in the Bahía Tortugas area during the spawning season of *Haliotis*, *Megastraea*, *Tegula*, and other gastropods (personal observation). To observe gonadal maturation, a slit was made on the third whorl of the shell with a high-speed drill or a fine file. Coloration of gonads (cream-colored in males and moss green in females) allowed gender de-

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termination. Through the slit we took samples for observation of the degree of gamete development.

### Induced Spawning

After 140 days of gonad conditioning, an attempt was made to induce spawning by submitting 12 snail specimens to abrupt temperature changes. The temperature shifts went from 19°C to 28°C and then reversed. The procedure was repeated a second time. Each temperature change was maintained for 1.5 hours.

### Sieving of Embryos and Larvae

Embryos were sieved with 236, 160, 140, and 100- $\mu$ m-pore-size Nytex mesh to remove organic waste. Sieving was done every 4 days throughout the experiment. Benthic post-larvae were fed microalgae (*Nannochloropsis oculata* Droop & Hibberd, 1977, and *Phaeodactylum tri-cornutum* Bohlin, 1974) in a 1:1 ratio. Observation and recording of morphological changes were made with a video monitor attached to a microscope. Seawater containing larvae and early juveniles was maintained at 17°C to 20°C.

## RESULTS

### Removal and Maintenance

Handling and transporting of adult *M. undosa* in coolers in a humid environment proved effective. No deaths occurred following 14 hours of transport. During the first 3 days in the laboratory, the specimens did not eat the rehydrated *Macrocystis pyrifera*, but accepted it later as a regular diet throughout the experiment.

### Induced Spawning and Fertilization

Induced spawning produced no immediate results. One week later, a spontaneous first spawning occurred. Two additional spawnings occurred, separated by 1-week intervals. The snails released gametes as pulses, raising the shell and spinning it rapidly from right to left with each gamete ejection. Fertilized eggs settled to the bottom of the tank where they were collected for transfer to culturing aquariums. For the uncontrolled spawning, polyspermy was prevented by sieving the embryos.

### Development

Observations were made from the time of fertilization through the early juvenile phase. The following descriptions identify the stages and the more prominent changes in the development of the species.

### Embryonic Development

**Day 1.** Fertilized eggs range in diameter from 160 to 180  $\mu$ m. The first polar body forms 30 to 45 min (1) after

fertilization. The second polar body appears 60 to 90 min after fertilization; immediately followed by the first cleavage, resulting in two same-sized cells. Subsequent cleavages occur at 90 to 120 min intervals (stage 2).

Morula development occurs at 3 to 4 hr, with diameters ranging from 170 to 190  $\mu$ m (stage 3). Ciliated gastrulae, 180 to 210  $\mu$ m in diameter, form at 9 to 10 hr and remain enclosed in the egg membrane (stage 4). Embryonic trochophores, 190 to 220  $\mu$ m in diameter, form at 12 to 13 hr (stage 5). Once the embryo becomes elongated, the prototrochal girdle begins to develop at one end (6). Later, two lateral tufts appear on the base (7).

The developing trochophore forms at 15 to 18 hr and is 190 to 230  $\mu$ m long. The prototrochal girdle and the lateral tufts provide a spinning motion on the longitudinal axis involving powerful, one-way movements in the direction of the anterior end of the embryo. When the trochophore breaks the enveloping membrane (8), it becomes a free-swimming trochophore (9). Shell secretion begins at the posterior end (10). Preveliger larvae (stage 11) occur when the larval shell (12) covers the posterior region of the trochophore at 22 to 24 hr.

**Day 2.** Early veligers (stage 13) are 190 to 230  $\mu$ m long. Veliger larvae, prior to torsion, extend the ciliary base of the velum (14) and there is notable growth of the cilia, as well as greater motility of the larva. The appearance of two grooves, resembling longitudinal lines, appears on the shell (15).

**Day 3.** Veliger larvae (stage 16) extend to 200 to 250  $\mu$ m in length. The fully formed shell covers the body near the base of the velum (17). After torsion, formation of the cephalo-pedal mass (18) and the operculum (19) begins. The velum becomes smaller and branches into two sections (20), which lengthen to form the lateral cephalic tentacles. The cilia of the velum loosen.

**Day 4.** Late veligers (stage 21). The foot displays retractile movements (22). Eye spots become apparent. The larvae exhibit exploratory movements, hurling themselves to the bottom of the container and swimming once more in the water column in search of attachment substrates.

### Benthic Phase

**Day 5.** The larvae range from 230 to 270  $\mu$ m, settle on the bottom to initiate the benthic phase (stage 23). Tentacles develop with six papillae each (24) and an aductor integument is present in the shell (25).

**Day 9.** Crawling post-larvae (stage 26) are 290 to 330  $\mu$ m long. Eyes are fully visible, cephalic tentacles have 10 papillae, vigorous active cephalo-pedal mass and amber-colored digestive gland are present. The anterior portion of the larval shell displays a suture (27), from which a new or teleoconch will begin to grow (28).

**Day 17.** Very active post-larvae reach 358 to 385  $\mu$ m in length (stage 29). They attach firmly to the substrate

Table 1

Development times in *Megastrea undosa* from embryo to juvenile under laboratory conditions, with temperature range of 17°C to 20°C.

Stage	Time
First polar body	30–45 min
Second polar body	60–90 min
Onset of cleavages	> 90–120 min
Morula	3–4 hr
Ciliate gastrula	9–10 hr
Embryonic trochophore	12–13 hr
Trochophore	15–18 hr
Preveliger	22–24 hr
Early veliger	2 day
Veliger	3 day
Late veliger	4 day
Settlement (benthic phase)	5 day
Crawling post-larva, secretion of larval shell	9 day
Post-larva, swelling of shell edges	17 day
Juvenile, crenulation of edges, elevation of teleoconch	30 day
Juvenile, crenulated whorl, brown pigmentation	45 day
Juvenile, heavily crenulated and whorled shell, dark pigmentation of cephalic region	70 day

and easily resist water disturbance. Swelling of the two edges or parallel ribs runs the length of the teleoconch, and its shape in cross section changes from cylindrical to quadrangular.

### Juveniles

**Day 30.** Juveniles (stage 30) reach shell length of 530 to 840  $\mu\text{m}$ . The cephalic portion of the shell becomes curved, elevating the whorl. There is greater calcification of the edges of the teleoconch (31). One edge begins to develop a crenulated ornamentation resembling the one in adult snails (32). Tentacles exhibit 12 to 14 papillae.

**Day 45.** Juveniles reach a length of 670 to 990  $\mu\text{m}$ . The first whorl is now distinctly formed. The spiny crenulated external edge of the shell becomes more pronounced. Pigmentation of the smooth edge of the teleoconch, in the form of brown spots, now begins.

**Day 70.** Juveniles attain a shell length of 1035 to 1464  $\mu\text{m}$ , and are distinctly curved upward and display little activity. The teleoconch rises and spiral growth is very evident, particularly in dorso-ventral view (stage 33). The crenulated edge (34) and the heavily colored smooth edge (35) are now easily recognized. Dark pigmentation begins to appear in the cephalic region.

Table 1 and Figure 1 summarize the various developmental stages and structures described above. The average rate of growth was 14.41  $\mu\text{m}\cdot\text{day}^{-1}$  (Figure 2).

### DISCUSSION

Maintenance of adult *M. undosa* specimens in the laboratory poses no major difficulties, since all specimens sur-

vived the 6 month test period. The wavy turban snail tolerates handling and adapts well to monospecific rehydrated algae diets. Gonad conditioning, however, required a long time when compared to laboratory studies of pectinids and venerids (Maeda et al., 1989; Tripp, 1985; Loosanoff, 1937). These two bivalves normally require 30 days of conditioning, while *M. undosa* attained its first spawning in 150 days. In the wild, *M. undosa* begins to spawn in September and reaches its reproductive peak in October/November (Belmar Pérez et al., 1991). Lengthy conditioning might be related to the degree of maturity of the specimens sampled, effects of feeding on a monospecific diet, and/or response to a static set of culture conditions.

Response to thermal treatment to induce spawning was not immediate, occurring 6 days after stimulation. Although spawning was spontaneous, we take it to be a late response, since untreated specimens did not spawn. Spontaneous spawnings at 1 week intervals in the stimulated specimens suggest that, once the gonad is conditioned, gamete release occurs in pulses. This agrees with observations of populations in natural settings, where the species is capable of behaving as a full- or partial-spawner, depending on environmental conditions (Belmar-Pérez et al., 1991).

Benthic diatoms are frequently used to feed post-larval gastropods during the benthic phases (Kawamura et al., 1998). In this experiment the pelagic microalgae *Nannochloropsis oculata* and *Phaeodactylum tricornutum* proved beneficial, allowing the specimens to develop up to the juvenile stage before switching to a macroalgae diet. Comparison of embryonic development with closely



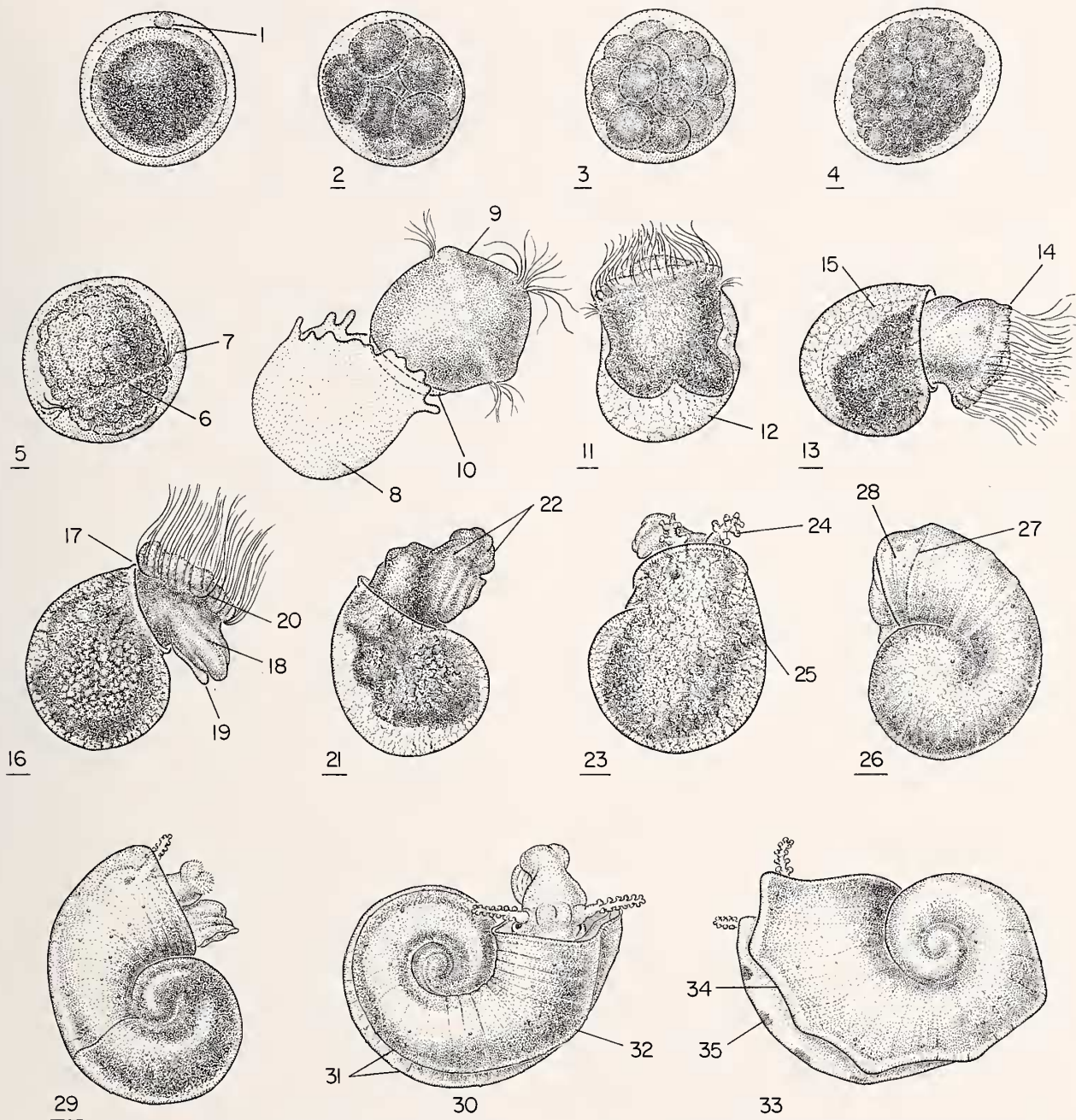


Figure 1. Developmental stages of *Megastrea undosa* from egg to 70 days. 1. Fertilized egg and formation of first polar body. 2. Cell cleavages (8 cells). 3. Morula. 4. Gastrula. 5. Formation of trochophore. 6. Prototrochal girdle. 7. Presence of cilia. 8. Hatching of trochophore larva. 9. Free trochophore. 10. Onset of larval shell formation. 11. Preveliger. 12. Larval shell. 13. Early veliger. 14. Development of the velum. 15. Longitudinal grooves. 16. Veliger. 17. Full shell covering the entire body. 18. Cephalo-pedal mass. 19. Operculum. 20. The velum splits into two masses. 21. Late veliger. 22. Retractable foot. 23. Benthic post-larva. 24. Cephalic tentacles with papillae. 25. Shell adductor integument. 26. Crawling post-larva. 27. Suture, marking the beginning of the teleoconch. 28. Teleoconch. 29. 17-day-old juvenile, development of teleoconch and initial swelling of edges. 30. 30-day-old juvenile. 31. Edges of the teleoconch. 32. Onset of crenulate ornamentation on one edge. 33. 70-day-old juvenile. 34. Crenulated edge. 35. Smooth pigmented edge.

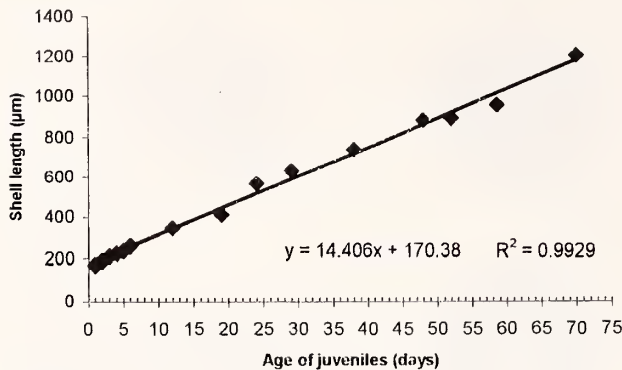


Figure 2. Post-larval and juvenile growth of *Megastraea undosa*. Day 0 corresponds to early veliger stage.

related species that occupy the same community reveals that the size of eggs, trochophores, and veligers is similar to the ones reported in other gastropods, such as *Haliotis* spp. (Sawatpeera et al., 2001) and *Tegula funebris* (Moran, 1997). Likewise, onset of the benthic phase in *M. undosa* occurs on day 5 of development, similar in fashion to *Haliotis* spp., which usually settle to the bottom between day 4 and day 5 (Mgaya, 1995).

The process of early developmental stages of *M. undosa* may help us to understand the life stages leading to recruitment in more detail, and eventually the dynamic population of this species and abalone, which constitute a significant part of the animal biomass of benthic communities in Baja California. Additionally, these data provide the groundwork for developing artificial cultures of *M. undosa*, as fishing pressure is becoming more severe. Culturing of the species may be necessary in the very near future.

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